

A/E 5/25/05

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**Amendments to the Claims:**

**Listing of Claims:**

1-44. (Cancelled)

45. (Previously presented): An isolated and purified endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity.

46. (Previously presented): The isolated and purified bovine endothelial cell growth factor of Claim 45 which comprises an unglycosylated form of a glycoprotein obtained from a culture of bovine folliculi stellate cells.

47. (Previously presented): The isolated and purified bovine endothelial cell growth factor of Claim 45 wherein the folliculi stellate cells are derived from a bovine source which factor comprises at its N-terminus the amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-.

48. (Previously presented): An isolated and purified bovine endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45 kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity.

49. (Previously presented): The isolated and purified bovine endothelial cell growth factor according to Claim 48 which comprises at its N\_terminus the amino acid sequence 'Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Gln-Lys-Pro-His-Glu.

50. (Previously presented): The isolated and purified bovine endothelial cell growth factor of Claim 49 which comprises at its N-terminus the amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-.

*Not Clear what he wants here*  
51. (Currently amended): The isolated and purified bovine endothelial cell growth factor of Claim 45 having an N-terminus amino acid sequence of  $\approx$  <sup>1</sup>Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His- Glu-Val-Val-Lys-Phe- Met-Asp-Val-Tyr-Gln-(Arg)-Ser-Phe-X-Arg-Pro-Ile-Glu-Thr-Leu-(Val)-X-Ile-X-(Gln)-Glu-Tyr- (Pro)-

wherein the amino acids in parenthesis are uncertain and the -X indicates an amino acid of unknown identity.

52. (Previously presented): The isolated and purified bovine endothelial cell growth factor according to Claim 45 which has a molecular weight of approximately 43Kd.

53. (Previously presented): The isolated and purified bovine endothelial cell growth factor according to Claim 52 which, under reducing conditions, forms two substantially homologous units, each unit having a molecular weight of about 23,000 daltons.

54. (Previously presented): An isolated and purified endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity, which factor further comprises an internal amino acid sequence, obtainable upon tryptic digestion, which sequence comprises Ser-Phe-Cys-Arg-Pro-Ile-Glu-Thr-Leu-Val-Asp-Ile-Phe-Gln-Glu-Tyr-Pro-Asp-Glu-Ile-; or Ser-Phe-Cys-Arg-Pro-Ile-Glu-Thr-Leu-Val-Asp-Ile-Phe-Gln-Glu-Tyr-Pro-Asp/Ile-Glu-.

*misplaced Modifier*  
55. (Currently amended): A The pharmaceutical composition for use with a human being comprising the isolated and purified bovine endothelial cell growth factor of claim 48 and a pharmaceutically -acceptable carrier by administration of said composition to a wound.

56. (Withdrawn): The method of promoting the proliferation of bovine endothelial cells in vitro which comprises applying to an effective amount of an isolated and purified endothelial cell growth factor to endothelial cells of Claim 45.

57. (Withdrawn): The method of Claim 56, wherein the endothelial cells are grown in cell culture.

58. (Currently amended): The pharmaceutical composition comprising the isolated and purified bovine endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity of Claim 45 and a pharmaceutically-acceptable carrier

*Comp Hybrid Claim* [ for promoting the proliferation of human endothelial cells in vivo, which includes: administering to such human cells an effective bovine amount of isolated and purified bovine endothelial cell growth factor. ]

59. (Currently amended): The pharmaceutical composition of Claim 58 wherein the carrier is a parenteral carrier

*Hybrid Claim* for promoting the proliferation of human endothelial cells in vivo which comprises:  
a) administering to a wound a pharmaceutical composition comprising bovine endothelial cell growth factor which comprises a protein which is obtained from bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity which factor comprises at its N-terminus the amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu.

60. (Currently amended): The pharmaceutical composition of Claim ~~59~~ 58 ~~which is adapted for in vivo wound healing in a human being by administering said bovine growth factor to a wound of a human being at a concentration of between about 10 picogram/milliliter and about 500 picogram/milliliter.~~

61. (Currently amended): An isolated and purified bovine endothelial cell growth factor having in vivo wound healing activity in a human which factor is obtained by a method of obtaining a substantially pure bovine endothelial cell growth factor, which method comprises:

- (a) providing folliculi stellate cells from a bovine source containing levels of endothelial cell growth factor;
- (b) extracting a solubilized portion of said cells;
- (c) partially purifying the endothelial cell growth factor from the extract using aqueous salt precipitation;
- (d) fractionating the purified extract using affinity chromatograph employing heparin moieties linked to an insoluble support as the stationary phase and employing a step gradient mobile phase of increasing salt concentration;
- (e) fractionating the extract using gel exclusion chromatography; and optionally
- (f) purifying the extract using reverse-phase high pressure liquid chromatography.

62. (Currently amended): The A method of use of the bovine endothelial cell growth factor of Claim 45 for ~~use in~~ the treatment of wounds by application to an in vivo surface wound.

63. (Currently amended): The method of use of the pharmaceutical composition comprising said bovine endothelial cell growth factor of Claim 60 for ~~use in~~ wound healing, by application to a surface wound.

64. (Previously presented): The isolated bovine endothelial cell growth factor obtained by a process for producing bovine endothelial cell growth factor comprising, providing a replicable expression vector capable of expressing the DNA sequence encoding human endothelial cell growth factor in a suitable host, transforming said host to obtain a recombinant host and maintaining said recombinant host under conditions permitting

expression of said endothelial growth factor- encoding cDNA sequence to produce the endothelial cell growth factor which is isolated.

65. (Previously presented): The isolated bovine endothelial cell growth factor obtained by said process of 64 wherein in the process said expression vector is selected from the group consisting of a bacteriophage and a plasmid.

66. (Previously presented): The bovine endothelial cell growth factor obtained by the process for producing bovine endothelial cell growth factor comprising, providing a replicable expression vector capable of expressing the DNA sequence encoding bovine endothelial cell growth factor in a suitable host, transforming said host to obtain a recombinant host and maintaining said recombinant host, under conditions permitting expression of said endothelial growth factor - encoding cDNA sequence to produce the endothelial cell growth factor which is isolated .

67. (Previously presented): The isolated bovine endothelial cell growth factor obtained by the process of Claim 66 wherein in the process said expression vector is selected from the group consisting of a bacteriophage and a plasmid.

68. (Withdrawn): A method of promoting the proliferation of human endothelial cells in vivo, which method comprises:

- (a) applying to endothelial cells a therapeutically effective amount of an isolated and purified endothelial cell growth factor wherein  
said endothelial cell growth factor is a protein obtained from bovine folliculi stellate cells in the form of a dimeric protein having a molecular weight of approximately 43–45 kd which protein is obtained by SDS PAGE under non-reducing conditions, and
- (b) obtaining in vivo endothelial cell growth.

69. (Withdrawn): The method of Claim 68 wherein in step (a) the protein further includes a pharmaceutically-acceptable carrier.

70. (Withdrawn): The method of Claim 69 wherein said protein is present in between about 10 picogram/ml and 500 picogram/ml.

71. (Withdrawn): The method of Claim 68 wherein said purified bovine endothelial cell growth factor forms two substantially homologous units each having a molecular weight of about 23 kd.

72. (Withdrawn): The method of Claim 68 wherein for the isolated and purified bovine endothelial cell growth factor the folliculi stellate cells are derived from a bovine cell source which factor comprises at its N-terminus the amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-.

73. (Withdrawn): The method of Claim 68 wherein for the isolated and purified bovine endothelial cell growth factor has an N-terminus amino acid sequence of - Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-(Arg)-Ser-Phe-X-Arg-Pro-Ile-Glu-Thr-Leu-(Val)-X-Ile-X-(Gln)-Glu-Tyr-(Pro)- wherein the amino acids in parenthesis are uncertain and the -X indicates an amino acid of unknown identity.

74. (Withdrawn): The pharmaceutical composition of Claim 68 for promoting the proliferation of bovine endothelial cells in vivo, which composition comprises:

(a) an isolated and purified endothelial cell growth factor wherein said endothelial cell growth factor is a protein obtained from bovine folliculi stellate cells in the form of a dimeric protein having a molecular weight of approximately 43-45 kd which protein is obtained by SDS PAGE under non-reducing conditions, which protein is administered in an effective amount to human tissue in need of wound healing.

75. (Withdrawn): The pharmaceutical composition of Claim 74 wherein in subpart (a) the composition further includes a pharmaceutically-acceptable carrier.

76. (Withdrawn): The pharmaceutical composition of Claim 74 wherein

in subpart (a) said protein is present in the composition in between about 25 picogram/ml and 500 picogram/ml.

77. (Withdrawn): The pharmaceutical composition of Claim 74 wherein said purified human endothelial cell growth factor is formed of two substantially homologous units each having a molecular weight of about 23 kd.

78. (Withdrawn): The pharmaceutical composition of Claim 76 wherein said protein is derived from a bovine cell source and comprises at its N-terminus the partial amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-.

79. (Withdrawn): The pharmaceutical composition of Claim 78 wherein the protein has an N-terminus partial amino acid sequence of <sup>1</sup> Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-(Arg)-Ser-Phe-X-Arg-Pro-Ile-Glu-Thr-Leu-(Val)-X-Ile-X-(Gln)-Glu-Tyr-(Pro)- wherein the amino acids in parenthesis are uncertain and the -X indicates an amino acid of unknown identity.